Center for Food Safety Engineering
2008 - 2009 Research Report

“Collaborating to make our food safer”
The mission of the Center for Food Safety Engineering is to develop new knowledge, technologies and systems for detection and prevention of chemical and microbial contamination of foods. Through CFSE, Purdue University positions itself as a national leader in multi-disciplinary food safety research. Our multi-disciplinary approach, including a strong engineering component, makes Purdue University truly unique.
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Welcome from
the Director

The Center for Food Safety Engineering (CFSE) at Purdue University celebrates our ninth year of partnership with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Eastern Regional Research Center (ERRC). Our collaborative efforts continue to advance the detection and control of food-borne pathogens and chemical hazards. This year, the CFSE team generated 25 peer-reviewed research publications and presented 15 talks at national science meetings.

This research report features results and progress from a number of projects. Our multi-disciplinary approach involves development of new technologies to improve detection of bacterial pathogens including *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Campylobacter* spp., and *Salmonella* spp. Engineers and food scientists are working together on food sample handling; biological target separation and concentration; and different detection platform systems including biosensor development, optical biosensors, cell-based biosensors, microarrays, infrared spectroscopy (including Fourier transform infrared spectroscopy), enzyme-linked immunosorbant assays, polymerase chain reactions, impedance-based microbiology, scanning microscopy, confocal raman microscopy, bioluminescence, DNA/RNA probes, and bioreporter-based chemical sensors.

Our team is making a greater impact internationally. This year, scientists presented their work in China, Hong Kong, Amsterdam, and Sweden. In November, two keynote presentations will be delivered from CFSE scientists at the International Association of Food Protection Meeting in Seoul, South Korea. We also look forward to visiting with guests from Jiao-Tong University in Shanghai, China, at our annual meeting this year.

Many of our technologies are entering the commercialization stage. The research team at Purdue that has developed the BActeria Rapid Detection using Optical scattering Technology (BARDOT) is collaborating with Advanced Bioimaging Systems, LLC to build the sensing units for industrial validation and use. Some of our researchers work with BioVitesse Inc., a biotechnology company that develops, manufactures, and markets automated in-process quality control monitoring systems and solutions for bacterial detection and identification. Most recently, Dr. Bruce Applegate co-founded Intelliphage, a company that develops methods for detecting food-borne pathogenic bacteria.

I continue to be impressed with the collaborative research efforts of Purdue University and USDA-ARS scientists and feel privileged to serve as director of the center. If you are interested in learning more about CFSE, please visit our Web site at www.cfse.purdue.edu or contact me directly.
As the collaboration between the CFSE at Purdue University and the USDA-ARS Eastern Regional Research Center continues, I am grateful to witness the continual growth, maturation, and research impact of this partnership. This partnership is considered an integral part of USDA-ARS research efforts. Together, we have received increased recognition as an important contributor to the technological advancement of pathogen detection in food—evidenced by the fact that the team received invitations from the International Workshop on Rapid Methods and Automation in Microbiology to conduct half-day symposiums on molecular methodologies in 2006, 2007, 2008, and 2009. The team received extraordinary praise from Dr. Daniel Y. C. Fung, Professor of Food Microbiology, Kansas State University, for invaluable contributions to the workshop, and he extended an invitation for our continuing involvement and celebration of its 30th anniversary in 2010. In 2008, our team went to Shanghai, China, to attend the first annual meeting of the Joint United States-China Food Safety Center, an international collaboration established between the ARS and Jiao-Tong University in Shanghai. This collaboration is part of the cooperative research activities between the USDA and the Ministry of Science and Technology, China (MOST). To signify this collaboration, high level officers of MOST and distinguished faculty members of Jiao-Tong University in Shanghai will attend our annual meeting in October this year. With this collaboration in place, I believe that our ARS-Purdue team together with our Chinese counterpart will become an important international research enterprise in the near future.
“New technologies for detecting foodborne pathogens that are rapid, sensitive, and portable with a potential for on-site detection are needed to ensure a safe food supply for consumers.”
Detection of foodborne pathogens via an integrated spectroscopy and biosensor-based approach

Project Rationale
Identification of microbial contaminants, such as pathogenic *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, is a primary food safety concern in food production, processing, and retail environments. Current detection methods for *E. coli* O157:H7 require enrichment for 18 to 24 hours followed by isolation, prescreening, and confirmation with classical biochemical methods or commercially available assays based on ELISA, antibody precipitation, or PCR. These procedures require up to four days to completely identify *E. coli* O157:H7. The infective dose for *Salmonella* strains varies with the server, food, and person. As few as one to ten cells can cause illness, and ranges from 1 to 10^7 CFU/ml of *Salmonella* strains have been reported.

New technologies for detecting foodborne pathogens that are rapid, sensitive, and portable with a potential for on-site detection are needed to ensure a safe food supply for consumers.

Project Objectives
- Develop and standardize Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy-based molecular fingerprints (spectra) of foodborne outbreak strains in conjunction with sampling and regulatory validation in food matrices.
- Advance infrared equipment, sampling, testing, and validation capabilities for rapid identification of foodborne pathogens.

Project Highlights
We completed a spectral library of Raman and FTIR fingerprints for *E. coli*, *Salmonella*, *Listeria*, *Shigella*, and *Staphylococcus*; Raman fingerprints were found to be sharper than the FTIR fingerprints. We classified key pathogens using chemometrics, and we classified outbreak strains.

Next, we developed a magnetic particle-based assay to separate a pathogen of choice, and the separated molecules were fingerprinted and detected by the portable spectrometer. This achievement represents the first portable IR-biosensor. We achieved highly selective detection in fewer than 30 minutes at both species (*E. coli* O157:H7 vs. *S. typhimurium*) and strain (*E. coli* O157:H7 vs. *E. coli* K12) levels in complex food matrices (two percent milk, spinach extract) with a detection limit of 10^4–10^5 CFU/ml. The combined approach of functionalized magnetic nanoparticles and IR spectroscopy imparts specificity through spectroscopic fingerprinting and selectivity through species-specific antibodies with a built-in sample extraction step. This approach could be applied in the field for on-site foodborne pathogen monitoring.
"This work can potentially have a significant impact on food safety by enabling the detection of foodborne pathogens using biochip-based point-of-test sensors."
Improved detection techniques for foodborne pathogens (development of biosensors component)

Project Rationale
We are using microfluidics and nanotechnology to develop rapid, sensitive, and selective biosensors for the detection of foodborne pathogens, such as *Listeria monocytogenes*. Our goal is to develop microfluidic biochips for rapid electrical detection of bacterial growth activity, and label-free electrical detection of nucleic acid molecules, especially Polymerase Chain Reaction (PCR) amplicons. This work can potentially have a significant impact on food safety by enabling the detection of foodborne pathogens using biochip-based point-of-test sensors.

Project Objectives
- Integrate molecular recognition through real-time PCR assays on the biochip. Use microfabrication techniques to assemble multi-channel configurations on the biochip, which will enable multiple assays to be performed.
- Develop label-free detection strategies to measure the progression of PCR with Dielectrophoresis (DEP)-based concentrations of nucleic acids using impedance-based detection of nucleic acid amplification.

Project Highlights
This year we demonstrated label-free electrical detection of PCR products using impedance measurement techniques. We were able to show that as little as 1 ug *L. monocytogenes* can be detected from cell lysate. We targeted the prfA 508 bp long gene using primers and measured the changes in the impedance of the solution with the amplified product after 25–30 cycles. We also detected *L. monocytogenes* in a mix of *Escherichia coli* O157:H7 and *Listeria innocua* in selectivity experiments using these techniques.
“The filtration-FTIR techniques have the additional potential advantages of discriminating between live and heat-treated E. coli O157:H7 cells...”
Improved detection techniques for foodborne pathogens (FTIR applications component)

**Project Rationale**
Viability assessment of bacteria is important in a wide variety of applications in the food industry, including evaluation of the effectiveness of inactivation treatments and microbial quality assessment of foods. The suitability of pathogen detection methods for food analysis depends on sensitivity, total time to detection, and cost of consumables. Even though the detection limit for conventional plating methods is low, the detection procedures are labor-intensive, often requiring extensive sample preparation and long incubation times. Conventional detection methods take at least 24 to 48 hours to differentiate and identify microorganisms; therefore, measures taken to counteract food contamination must wait at least that long. Additionally, conventional methods do not provide information about dead bacteria and may underestimate sub-lethally damaged cells present in a food sample.

**Project Objectives**
- Improve Fourier-transform infrared spectroscopy (FTIR) detection techniques for pathogen detection.
- Identify detection limits for methods developed and opportunities for improving sensitivity and specificity of FTIR detection techniques.
- Determine how spectroscopic differentiation relates to known cell surface characteristics from laboratory and foodborne outbreak pathogens.

**Project Highlights**
FTIR pathogen detection techniques were improved this year by determining how spectroscopic differentiation relates to cell surface characteristics, how these structures vary with conditions and treatments, and how the techniques could be applied to detect pathogens in food systems (fruit juice, ground beef, and chicken breast). In studies using *Escherichia coli* O157:H7, the influence of different types of inactivation treatments (sterilization at 121 °C, UV light, alcohol, sodium chloride, chloramphenicol, and trimethoprim-sulfamethoxazole) on spectra was determined. The FTIR technique can be used to discriminate bacteria subjected to different inactivation treatments and possibly to assess the degree of cell injury caused by each treatment. FTIR methods were able to differentiate live and dead cells of *E. coli* O157:H7 as well as quantify live cells of *E. coli* O157:H7 in the presence of dead cells ($R^2 > 0.996$).

Coupling separation techniques (filtration and Dynabeads®) with FTIR analyses enabled the detection and quantification of *E. coli* O157:H7 from ground beef. The filtration-FTIR and Dynabeads®-FTIR approaches are relatively faster and less expensive than many other techniques used for this purpose. The filtration-FTIR techniques have the additional potential advantages of discriminating between live and heat-treated (dead) *E. coli* O157:H7 cells, differentiating between pathogenic and non-pathogenic *E. coli* strains, and quantifying live cells in the presence of dead cells.
“The availability of rapid methods for detecting pathogens in food production, processing, and distribution systems could enable the real-time assessment of risks.”
Improved detection techniques for foodborne pathogens (multi-plex detection platforms component)

Project Rationale
Microbial contamination of meat and, more importantly, fresh fruits and vegetables has become a mounting concern during the past decade due to an increased emphasis of these products in a healthy diet and the recognition of new foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. The availability of rapid methods for detecting pathogens in food production, processing, and distribution systems could enable the real-time assessment of risks. However, the speed of the method is usually indirectly correlated with the cost (less time, more money). The prime example is nucleic acid amplification technologies, which can provide rapid quantitative results, but instrumentation costs can exceed one hundred thousand dollars. Therefore, this effort is focused on the development of a low-cost nucleic acid amplification detection platform that can quickly screen for multiple pathogenic microorganisms and can be operated in the field by non-technical personnel.

Project Objectives
- Integrate a peltier heating/cooling device into the previously designed spatial beacon format.
- Define the parameters needed for utilizing mRNA from previously determined amplicons to differentiate between live and dead cells.
- Develop an improved low-cost optical detector system for analyzing the dedicated DNA microarray FRET-probe platform.
- Develop optimized protocols for the developed platform.

Project Highlights
We developed a prototype integrating a peltier heating/cooling device into the previously designed spatial beacon format. The basic functions of the prototype device are to amplify the gene region of interest using common thermocycling methods (i.e. PCR), and to detect a color change in the fluorescence of the capture probe. The system is capable of quickly heating/cooling the sealed reaction chamber, which we prototyped using a microscope slide ‘sandwich’ with a small gasket between them.

We achieved the heating/cooling functions using peltier thermoelectric modules adhered to a thin aluminum plate placed against the microscope slide sandwich. The heating/cooling system was controlled by using an integrated thermocouple at the microscope slide surface, which provides real-time temperature readings from the reaction chamber. This feedback loop (heating/cooling signals to the peltier modules and reaction chamber temperatures from the thermocouple) provided the basic input/output for the development of a control software system and supporting circuit board. Initial PCR reactions analyzed by gel electrophoresis validated the prototype’s performance. Currently, we are interfacing the prototype thermocycler with a CCD-based optical detection system, as well as a software-controlled laser-based excitation system, and are conducting preliminary tests.

Bruce Applegate (Department of Food Science)
“Multiple detection technologies coupled with target separation from food matrices provide the necessary breadth of orthogonal methods that ensure detection of a diverse set of pathogens...”
**Improved detection techniques for foodborne pathogens (separation techniques component)**

**Project Rationale**

The long-term objectives of this project are to develop, validate, and implement new technologies and systematic approaches for detecting microbial and chemical contamination of foods. The approach focuses on four main components including separation, detection, identification, and quantification of target microorganisms from food matrices. The rapid and accurate detection of foodborne pathogens in meats, dairy products, and vegetables is a critical component for achieving food safety, preventing deaths and severe illnesses caused by foods, and minimizing economic losses to the food industry. Recalls of food products may be minimized by catching any problems before the products leave the plant. Rapid detection is a powerful tool in achieving such a goal.

Our research addresses cell separation and concentration technologies to facilitate pathogen detection and the development, validation, and implementation of robust new identification and quantification technologies. Multiple detection technologies coupled with target separation from food matrices provide the necessary breadth of orthogonal methods that ensure detection of a diverse set of pathogens, and provide platforms that facilitate both detection and identification of pathogens. Our overall goal is to develop robust operational technologies for the food safety industry, regulatory inspection, and food safety research.

**Project Objectives**

- Characterize the pore structure of hollow fiber membranes with respect to the potential to entrap microorganisms and particles in the one to two micron range against a background of small particulates derived from food materials.
- Identify the particle size of microbial adsorbents. Demonstrate the adsorption of microorganisms from food matrices, and the separation of particles from homogenized food matrices.
- Inoculate GFP-expressing microorganisms into food, recover the microorganisms, and image them using fluorescent microscopy and SEM.
- Achieve a multiplexed DEP/Ab mediated capture of *Escherichia coli* and *Listeria monocytogenes* in the same microfluidic biochip.

**Project Highlights**

We found that the distribution of cells between solid food particles and a surrounding liquid (aqueous buffer) concentrates cells on the surface of food particles. We hypothesize that this effect is a function of pH and ionic strength, since this would impact the surface charge (zeta potential) of cells, as well as the charge of the proteins on the surface of the particles. Like charges would release cells, while unlike charges would attract them (causing adsorption). We are systematically examining the effects of buffer properties (ionic strength, pH, and ionic species) to obtain devices that are capable of releasing cells from food particles and then utilizing them for subsequent interrogation using PCR, fluorescent labeling, fourier-transform infrared spectroscopy, and other methods.
“We developed a sandwich immunofluorescence assay in a microarray format for capturing and detecting L. monocytogenes, E. coli O157:H7, and S. Enteritidis...”
Multipathogen screening using immunomicroarray

**Project Rationale**
Most assay methods for detecting pathogenic foodborne bacteria are developed for the detection of a single target pathogen. As a result, these methods can be costly when testing for multiple pathogens from a single sample because separate assay methods are required. The overall objective of this project was to develop a 96-well plate-based sensitive immunoassay for concurrent detection of three pathogens—*Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Enteritidis—occurring simultaneously in a test sample. Multi-pathogen analysis on a single assay platform will eliminate the need for separate tests for multiple microorganisms, reduce test costs, and ultimately improve food safety.

**Project Objectives**
- Develop a microarray assay in a 96-well plate and glass slide using a sandwich immunoassay for three pathogens.
- Optimize growth and enrichment of three pathogens (healthy or stressed) spiked in model food samples in a selective enrichment broth for use with the microarray.
- Evaluate the performance of the Pathogen Enrichment Device.

**Project Highlights**
We developed a sandwich immunofluorescence assay in a microarray format for capturing and detecting *L. monocytogenes*, *E. coli* O157:H7, and *S. Enteritidis* using antibodies developed in our laboratory. The different antibodies for each pathogen were screened for their efficiency either as capture or detection antibodies. Streptavidin-coated microtiter plates were used to immobilize biotinylated antibodies for the detection of target pathogens. For *L. monocytogenes*, we used biotinylated-P66 as a capture antibody, and horseradish peroxidase or Alexa Fluor (AF)-labeled C11E9 as a detection antibody. We used biotinylated-anti-*E. coli* polyclonal Ab and AF-labeled-pET32a antibodies for *E. coli*, and biotinylated-anti-*Salmonella* PAb and AF-labeled MAb-2F11 antibodies for *S. Enteritidis*. These antibody combinations were applied to a sandwich fluorescent immunoassay in 96-well plates. The detection limit was determined to be 10⁵ cells/well. We have demonstrated that this 96-well microplate-based immunoassay can be used for specific detection of *L. monocytogenes*, *E. coli* O157:H7, and *S. Enteritidis* when grown in ready-to-eat meat products such as beef, chicken, and turkey.

Arun Bhunia (Department of Food Science)
“We are capitalizing on the unique spectroscopic signatures of non-fluorescing molecules as labels to identify specific DNA sequences.”
Nanoparticle-based DNA-multiplexed probes for pathogen detection using confocal raman microscopy

Project Rationale
The overall goal of this research is to develop a probe fabrication and assay synthesis protocol for multiplex-DNA detection of food pathogens by surface-enhanced raman scattering (SERS) utilizing non-fluorescent, label-containing nanoparticles as DNA probes. Although research on SERS-labeled DNA examination is very active, it is still in its early stages with regard to multiplexing and detecting analytes at low levels. We are capitalizing on the unique spectroscopic signatures (down to ~1 nm resolution) of non-fluorescing molecules as labels (raman tags) to identify specific DNA sequences. Because of the distinct fingerprint of the labels due to SERS, simultaneous detection of multiple DNA hybridizations without separation is feasible at sub femtomolar (fM) sensitivity.

Several aspects are unique to this research. We can use multiplex labeling in one system using a range of non-fluorescing labels. A one-pot platform for detection of food pathogens at sensitivities not afforded by fluorescence methods is possible using our approach. Incorporation of a magnetic separation step will enable the separation of target sequences in complex media. Using non-fluorescent labels (~$10–20/g) for multiplexing is many orders cheaper than fluorescent labels (~$10–20/mg). Furthermore, the choice of SERS labels is enormous (over 1,000 labels) and extremely sensitive, and single-molecule identification has been reported. This implies that eventually the detection can be accomplished without the amplification step.

Project Objectives
- Investigate the effectiveness and efficiency of two widely available fluorescent and five cheaper non-fluorescent dyes as raman labels to be used as SERS tags.
- Develop a one-pot multiplex detection system using the optimized SERS-DNA probe to simultaneously detect genes related to pathogens.

Project Highlights
Using the SERS method, we developed an eight-plex strategy to detect up to eight DNA interactions simultaneously. The raman labels we chose were non-fluorescent in nature; hence the detection cost is about 1,000 times cheaper than conventional fluorescence methods. In addition to detection, quantification of gene fragments was also possible at a level of sensitivity reaching 1 fM. This was accomplished in an array format. We also developed a tubeformat eight-plex detection scheme using non-fluorescent labels. We used this scheme to detect oligonucleotides depicting the three key pathogens. This proof-of-concept study demonstrates that the methods we developed can be used to detect and quantify genetic fragments extracted from multiple pathogens.
Arun Bhunia Honored by Institute of Food Technologists

May 4, 2009 | Institute of Food Technology

Arun Bhunia, was selected as the winner of the 2009 Research and Development Award by the Institute of Food Technologists (IFT).

The Research and Development Award is given yearly to an IFT member or team of members who have made a recent, significant research and development contribution to the understanding of food science, food technology, or nutrition. The award was presented at IFT’s Annual Meeting and Food Expo in Anaheim, CA on June 6th 2009. The Research and Development Award includes a plaque and a $3,000 honorarium from IFT.

Bhunia’s research has focused on early detection of food borne pathogens to reduce the risk of food borne illness outbreaks. Bhunia and his laboratory group have developed several biosensor tools for on-site testing of food products for food safety and food defense applications. Bhunia and two colleagues also created a laser light-scattering instrument for detection and identification of bacterial colonies in food.

Purdue scientists develop test to detect food safety

March 31, 2009 | WTHR Indianapolis

In Dr. Arun Bhunia’s lab a team of about a dozen people over eight years developed a way to detect listeria in 4-8 hours. Tests currently take 1-4 days.

“It is definitely a big deal,” said Dr. Bhunia, a professor of Food Microbiology. But how does it work?

Extracted fluid from a food sample is run through a chip. The harmful microorganisms bind to receptors, then grow. That is measured to determine how much of the organism is in the sample. A microscope confirms the levels.

Researchers hope to expand the test for use with E. coli and salmonella.

“We are very excited about the potential of this technology,” Dr. Bhunia said.

It is technology food processors could use, along with federal inspectors. But scientists say we are still a long way from a simple swap that would let consumers know if those lingering leftovers are safe.
Food safety recalls suggest need for more frequent and better testing methods, researcher says.

A New Test Is Quicker for Finding Tainted Food

A group of researchers at Purdue University are developing a new test to detect foodborne pathogens that can detect food poisoning. The test is based on a technique called optical biosensing, which detects the presence of specific pathogens directly from food samples.

The test works by detecting the presence of certain proteins on the surface of foodborne pathogens. These proteins are then detected using a combination of optical and chemical techniques. The test is designed to be faster and more sensitive than current methods, allowing for more rapid detection of foodborne pathogens.

The researchers hope that their test will eventually be used in food safety laboratories and in the field, allowing for quicker detection of foodborne pathogens and reducing the risk of outbreaks.

The test was developed by a team of researchers led by Dr. Bruce Applegate, a Purdue University professor of food science and technology. Applegate says that the new test could be used to detect foodborne pathogens in a wide variety of foods, including meats, fruits, and vegetables.

The test was tested on a variety of different foods, including chicken, beef, and vegetables. The researchers found that the test was able to detect foodborne pathogens at concentrations as low as one pathogen per gram of food.

The researchers are now working to optimize the test for use in food safety laboratories. They hope that the test will eventually be approved by the U.S. Food and Drug Administration (FDA) and become a standard part of food safety testing.

"This test is a significant step forward in the detection of foodborne pathogens," says Applegate. "It has the potential to revolutionize the way we test for foodborne pathogens and protect public health."
“We developed and optimized a detection method using an antibody-coupled fiber optic biosensor for the simultaneous detection of L. monocytogenes, E. coli O157:H7, and S. Enteritidis.”
Optical biosensors for food pathogen detection

Project Rationale
In this project, our ultimate goal was to develop a sensitive fiber optic-based sensor for the concurrent detection of multiple foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Salmonella* enterica serovar Enteritidis in a single food sample. In previous work, we were able to develop separate fiber optic sensor-based assays specific for each target pathogen: *L. monocytogenes*, *E. coli O157:H7*, and *S. Enteritidis*. During this project cycle, our goal was to optimize the fiber optic biosensor platform for the simultaneous detection of all three pathogens in a single assay format. This will not only reduce the cost per test, but will also ensure product safety with regards to the three key pathogens.

Project Objectives
- Develop and evaluate an antibody-coupled fiber optic biosensor (ANALYTE 2000™) for detecting *L. monocytogenes*, *E. coli O157:H7*, and *S. Enteritidis*.
- Validate the multiplex fiber optic sensor with inoculated ready-to-eat (RTE) shredded beef, chicken and turkey meat.

Project Highlights
We developed and optimized a detection method using an antibody-coupled fiber optic biosensor (ANALYTE 2000™) for the simultaneous detection of *L. monocytogenes*, *E. coli O157:H7*, and *S. Enteritidis*. For the capture of each bacterium, a biotinylated polyclonal antibody was immobilized on the fiber. We labeled each bacterium-specific monoclonal antibody with Alexa Fluor 647 and used it as a reporter antibody. The biosensor detected *L. monocytogenes*, *E. coli O157:H7*, and *Salmonella Enteritidis*. The limit of detection was found to be $10^3$–$10^4$ CFU/ml in pure cultures and in mixed culture conditions when inoculated into RTE meat such as beef, chicken, and turkey, which were enriched in a selective enrichment broth for *Salmonella*, *Escherichia*, and *Listeria*.
“We demonstrated that BARDOT can identify and differentiate microcolonies of Listeria innocua and E. coli...”
Optical forward scattering for bacterial colony differentiation and identification

**Project Rationale**
We are in the process of refining our BActeria Rapid Detection using Optical Technology (BARDOT) system to locate, capture, and classify foodborne pathogenic bacteria. The system requires a human operator to position the bacterial colony to the incident laser beam and adjust it such that the forward-scattering patterns are rotationally symmetric. This task is an iterative process and requires constant operator attention. By automating the instrument operation, we will increase its efficiency and reduce the time required for bacterial identification. In this reporting period, we concentrated our efforts on developing a fully automated detection and identification system which integrates the hardware and software of the forward-light scatterometer with the in-house classification software package. In addition, we tested the BARDOT system’s ability to detect microcolonies at the 100 to 200 μm diameter range (which, if successful, would substantially reduce the time required for bacterial growth and, therefore, time-to-result for test samples), and to detect pathogens in cooked and raw food samples.

**Project Objectives**
- Experiment with bacterial colony scattering at colony diameters of less than 500 μm.
- Acquire scatter images of colonies of select foodborne bacteria on non-selective and selective agar media.
- Test the BARDOT system’s ability to detect foodborne pathogens from artificially contaminated meat, vegetables, and seafood samples.

**Project Highlights**
We demonstrated that BARDOT can identify and differentiate microcolonies (at approximately 173 micrometerum in diameter) of *Listeria innocua* and *E. coli* after only eight hours of growth on an agar plate. Furthermore, laser triangulation sensors provided some profilometric data that show differences in colony morphology for different bacterial species.

In addition, we have generated scatter signatures for 14 serovars of *Salmonella*, 100 serotypes of *Escherichia coli* O157:H7 and other serotypes, and several other bacterial cultures to expand our scatter image library. Finally, we have shown that BARDOT can be used for detecting *Salmonella* from inoculated peanut butter and tomato, *E. coli* O157:H7 from ground beef and spinach, and *Listeria monocytogenes* from inoculated hotdog and milk samples, even after a prolonged storage at very low concentrations.

Arun Bhunia (Department of Food Science)
The device would offer a commercial advantage to the food processing industry.
Peptide array biosensor for high throughput and multiplexed detection of foodborne pathogens

Project Rationale

The increased incidence of pathogen-contaminated food places a new emphasis on the rapid detection and quantification of foodborne pathogens. Conventional pathogen detection methods involve enriching the sample and performing various media-based metabolic tests. These detection methods are elaborate and typically require two to seven days to obtain results. Therefore, we are developing a surface plasmon resonance (SPR) imaging biosensor for the rapid, label-free, high throughput detection of foodborne pathogens. This device integrates an SPR imaging system with a biosensor array immobilized onto the sample surface containing specific short peptide ligands. A group of short peptides, identified from phage display libraries and specific to certain pathogenic bacteria, will be microcontact-printed on a gold chip in linear patterns. This peptide-imprinted gold chip functions as a biosensor array for the specific detection of unknown foodborne pathogens. To determine what fraction of pathogenic bacteria are live or dead and to confirm the SPR results, we have created a novel hybrid SPR/molecular imaging portable system. The device would offer a commercial advantage to the food processing industry. It is miniaturized, has fewer components, and is easier to use compared to the current detection systems. This biosensor could detect foodborne pathogens present in <100 CFU/g of contaminated food within ten minutes.

Project Objectives

• Synthesize and characterize peptides.
• Fabricate and characterize the peptide biosensor array.
• Design and assemble a compact SPR imaging device.
• Achieve real-time detection of foodborne pathogens by SPR imaging.
• Optimize the device for high throughput and multiplexed detection.
• Use antibodies instead of peptides to capture pathogens.

Project Highlights

We have designed a hybrid microfluidic biochip to perform multiplexed detection of single-celled pathogens using a combination of SPR and fluorescence imaging. The device consists of an array of gold spots, each functionalized with a capture biomolecule targeting a specific pathogen. This biosensor array is enclosed by a polydimethylsiloxane microfluidic flow chamber that delivers a magnetically concentrated sample to be tested. The sample is imaged by SPR on the bottom of the biochip and epi-fluorescence on the top. This prototype instrument was able to image antibody-captured Escherichia coli O157:H7 bacteria by SPR and fluorescence imaging.

The efficiency of capture of these bacteria by the magnetic particles was determined using spectrophotometric ferric oxide absorbance measurements. We used NIH ImageJ software to measure the percent of the gold spot area upon which the E. coli was bound. This hybrid imaging approach of pathogenic E. coli detection coupled with an estimate of relative infectivity was shown to be a working example of a testing device for potential foodborne pathogens.
“Our research efforts will lead to the development of a small, portable, user-friendly detection device for regulatory agency representatives and other interested parties to quickly track food products having pesticide residues that exceed the legal limit.”
Portable biosensor for rapid and ultra-sensitive identification of organophosphorous foodborne contaminants

**Project Rationale**

Pesticides present serious risks to the environment, food supply, and public health due to their acute toxicity, potential carcinogenic effects, and mutagenic activities. Among pesticides, organophosphorus (OP) and carbamate (CB) compounds are the most used, representing 40 percent of the world’s pesticide market. These compounds are currently detected using elaborate reference analytical methods which are sensitive and reliable, but expensive, time-consuming, and ineffective at determining the pesticides’ toxic effects. Furthermore, their use is ineffective in situations when unpredictable contamination could suddenly appear in the environment and/or food supply. In such situations, general screening of samples using time-effective, low-cost, user-friendly devices would be a better strategy to detect toxicity. Our project employs this general strategy by using enzymes as biosensors that respond to the presence of OP and CB and signal if any enzymes in the sample are affected by these contaminants.

**Project Objectives**

- Establish the effect of immobilization on AChE and the need and feasibility of using an oxidation strategy for phosphorothionates.
- Fabricate and characterize the AChE biosensor by immobilizing the enzyme onto the surface of single-use screen-printing electrodes. Study enzyme stability and leaching.
- Detect pesticides. Obtain calibration plots of the inhibitory degree upon application of various concentrations of pesticides. Determine the detection limit, response time, and linear concentration range for selected pesticides.

**Project Highlights**

Our research efforts will lead to the development of a small, portable, user-friendly detection device for regulatory agency representatives and other interested parties to quickly track food products having pesticide residues that exceed the legal limit. This proposed biosensor would accelerate the testing procedure and provide useful screening results, even before a sample is sent to a central laboratory for testing; reduce analysis costs; increase the number of samples tested and screened near the field, and ultimately; increase food safety. Specifically, the proposed biosensor would detect and screen for OP pesticide residues using a simple-to-use streamlined method.
“The technology can significantly benefit the food industry by enhancing the laboratory-testing capabilities of food manufacturers and food testing laboratories as well as field-testing activities of governmental agencies.”
Rapid, quantitative, and reusable immunosensors for bacteria detection on a microfluidic platform

Project Rationale
Portable, rapid, and sensitive biosensors for food safety applications enable point-of-care contamination detection and immediate interpretation of the results. In our research project, we proposed to develop an integrated biosensor system on a microfluidic chip for detecting bacteria based on immunoassays. The device will offer a detection sensitivity of $10^2$ to $10^3$ bacteria cells and an assay time of fewer than 20 minutes for a single test. Our system will yield quantitative data for estimating the number of the target bacterium in a food sample. The microfluidic system will consist of individual devices for cell lysis, lysate purification, and immunoassays. In principle, the tool will be effective for any bacterium or strain given the availability of a suitable intracellular antigen-antibody pair. In this project, we will demonstrate the concept using an intracellular antigen, alcohol acetaldehyde dehydrogenase (Aad), and its monoclonal antibody (MAb-H7) to detect *Listeria monocytogenes*. In order to concentrate *L. monocytogenes* cells from food samples, we will fabricate magnetic nanobars with different sizes and geometries and develop protocols for immobilizing antibodies specific to *L. monocytogenes* on the surface.

A portable, reusable, and low-cost device would be useful for point-of-care analysis in the food manufacturing industry. Conducting bacteria detection tests within food manufacturing laboratories would dramatically decrease the turnaround time for the results and avoid potential contamination and changes in the bacteria during transit. Conventional analytical methods require bulky, expensive equipment that is often cost-prohibitive for food manufacturing laboratories. With our lab-on-a-chip approach, sophisticated functions of a biological laboratory can be miniaturized on a microchip, enabling any minimally equipped laboratory to perform bacteria detection tests. The technology can significantly benefit the food industry by enhancing the laboratory-testing capabilities of food manufacturers and food testing laboratories as well as field-testing activities of governmental agencies.

Project Objectives
- Fabricate magnetic nanobars with different sizes and geometries and develop protocols for immobilizing antibodies specific to *L. monocytogenes* on the surface. The amount of bacterial cells bound to the surface will be characterized under different conditions.
- Develop an electrophoresis-based immunoassay coupled with laser-induced fluorescence on a microfluidic chip. We will use this tool to quantitatively detect *L. monocytogenes* based on cell lysate via the interaction between Aad and MAb-H7.
- Demonstrate a prototype integrated microfluidic system which incorporates different steps such as manipulation of magnetic nanobars, cell lysis, lysate purification, and immunoassay.

Project Highlights
We have been developing an on-chip immunoassay that detects an intracellular antigen of *L. monocytogenes* (Aad) based on polystyrene beads functionalized with Aad antibody. We manipulated these beads in the microfluidic channel so that they were mixed thoroughly with the cell lysate. After binding with the antigen in the lysate, the beads were exposed to fluorescently labeled Aad. The detected bacterial concentration was inversely proportional to the fluorescence intensity from the beads after washing. Our detection method and device will be useful generally for immunoassays based on cell lysate. Such an approach will be important for developing portable microchip sensors for food safety applications.


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**Proceedings (2008-2009)**


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